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Morphological and genetic variation in Mexican wild populations of *Tamarixia radiata* (Hymenoptera: Eulophidae)

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Abstract

We analyzed the morphological and genetic variation of the Asian citrus psyllid nymphal ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) in 2 regions of Mexico, in the northeast (represented by the states of Nuevo León and Tamaulipas) and the west (represented by the states of Colima and Michoacán). We found that the morphological variation of the specimens lay mainly in body color traits. The morphometric study indicated that in comparison with females, males presented heterogeneity, and it was concentrated in the state of Colima. Despite the morphological variation found in the species, it was not exclusively associated with any of the geographical regions. Molecular analysis revealed the presence of 2 haplotypes (H1 and H2), which were the same found in previous research among strains introduced to Florida. Haplotype H2 was found in both studied regions and more frequently than haplotype H1, which was collected only in the northeast (Tamaulipas state), suggesting possible points of gene flow between Mexico and the USA. Our results have implications for the extensive use of *T. radiata* in biological control programs of the Asian citrus psyllid.

Key Words: morphology; morphometry; genetic variation; haplotypes

Resumen

En el presente estudio se analizó la variación morfológica y genética del ectoparasitoide ninfal del psílido asiático de los cítricos *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) en dos regiones de México, región noreste (representada por los estados de Nuevo León, y Tamaulipas) y región occidente (representada por los estados de Colima y Michoacán). Se encontró que la variación morfológica de los especímenes analizados recae principalmente en variables cromáticas. En el estudio morfométrico se encontró que a diferencia de las hembras, los machos presentan heterogeneidad, la cual se concentró en el estado de Colima. La variación morfológica en el parasitoide no se asoció de forma exclusiva por alguna de las zonas geográficas de estudio. El análisis molecular reveló la presencia de dos haplotipos (H1 y H2), los cuales se han encontrado en investigaciones previas entre las poblaciones del parasitoide introducidas a Florida, E.U.A. El haplotipo H2 se encontró en todas las áreas de estudio y con mayor frecuencia que el H1, el cual se localizó solamente en la región noreste (estado de Tamaulipas), sugiriendo posibles puntos de flujo génico entre México-E.U.A. Los resultados poseen implicaciones para el uso extensivo de *T. radiata* en los programas de control biológico del psílido asiático de los cítricos.

Palabras Clave: morfología; morfometría; variación genética; haplotipos

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) is a nymphal ectoparasitoid of the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Chien et al. 2001). The Asian citrus psyllid is the vector of '*Candidatus* Liberibacter asiaticus' (CLas), the putative causal agent of huanglongbing, a deadly disease for citrus (Gottwald et al. 2007). *Tamarixia radiata* is considered one of the main biological control agents of *D. citri* (Étienne & Aubert 1980). Both species have an Asian origin, and their wild forms have been found in different citrus-growing regions of the world (French et al. 2001; Cáceres & Aguirre 2005; González et al. 2007; Lizondo et al. 2007; Pluke et al. 2008; Gómez-Torres 2009; Boykin et al. 2012). Due to the high parasitism rates observed in its origin region, *T. radiata* was imported to several countries in an attempt to control *D. citri* and the spread of CLas (Quilici 1986; Chiu et al. 1988; Étienne et al. 2001; Aubert 2008).

The first classical biological control program with *T. radiata* began in 1978 in Réunion Island, with specimens introduced from Punjab, Pakistan (Étienne & Aubert 1980). Following the successful results obtained in Réunion, releases of *T. radiata* were made on Mauritius Island, in Taiwan, and in Guadeloupe from the colonies established in Réunion (Quilici 1986; Chiu et al. 1988; Étienne et al. 2001). In 1998, parasitoids from Taiwan and South Vietnam were imported to Florida, USA. (Hoy 1998; Hoy et al.1999). These parasitoids were kept under quarantine and released during the years 1999 to 2001 (Skelley & Hoy 2004); the degree of parasitism obtained was low (Michaud 2002). Strains of the *T. radiata* from

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southern China, Pakistan, and North Vietnam were later imported into Florida and studied under quarantine; there were no significant morphological differences among such quarantined strains (Barr et al. 2009).

Accidental introductions of *T. radiata* were registered in subsequent years in different countries and regions of the Americas, including Texas (French et al. 2001), Cuba (González et al. 2007), Brazil (Gómez-Torres 2009), Argentina (Cáceres & Aguirre 2005; Lizondo et al. 2007), Puerto Rico (Pluke et al. 2008), Venezuela (Barr et al. 2009), Mexico (Baeza-Nahed 2008; López-Arroyo et al. 2009; González-Hernández et al. 2010), and Colombia (Kondo et al. 2012). In some countries, molecular studies using the cytochrome oxidase subunit I gene were performed, finding diverse haplotypes and morphological variants attributed to environmental factors (Barr et al. 2009; Gómez-Torres 2009; González-Hernández et al. 2010). Some of the identified haplotypes appear to have originated in strains from China, Taiwan, and Vietnam (Barr et al. 2009; De León & Sétamou 2010; González-Hernández et al. 2010).

The discovery of wild populations of *T. radiata* in Mexico prompted the government to take advantage of its presence and adopt its use for the biological control of *D. citri* in a national program. Since 2008, mass rearing and release technologies have been developed in order to fulfill the demand for *T. radiata* in huanglongbing-affected areas (López-Arroyo et al. 2009; Sánchez-González 2011). Variability of the species in Mexico is unknown, and certain kinds of variations could hamper the success of the strains that have been used in the country. In this research, we characterized the morphology and genetics of *T. radiata* specimens collected from two Mexican regions. The goal was to describe the diversity of *T. radiata* to determine the existence of distinct strains or unusual geographic populations of the parasitoid, which could improve the use of this biological control agent. Furthermore, knowledge of forms, varieties, or haplotypes corresponding to specific geographical regions would be useful to evaluate hypotheses regarding the introduction and establishment of the species in the country.

Materials and Methods

We used *T. radiata* specimens from 2 representative regions of Mexico, the northeast (represented by the states of Nuevo León and Tamaulipas) and the west (represented by the states of Colima and Michoacán). The parasitoids were collected with entomological nets or from parasitized nymphs. The samples from the northeast were collected during 2006, 2007, and 2010 whereas those from the west were collected during 2010. In addition, some samples were provided by colleagues of the national project FONSEC SAGARPA-CONACYT 2009-108591. This research was conducted in 3 phases: morphological characterization, morphometric characterization, and molecular analysis.

MORPHOLOGICAL CHARACTERIZATION

Fifteen characters localized in the head, antennae, thorax, abdomen, wings, and legs were examined (Table 1). Most of the traits were chosen based on previous studies (Waterston 1922; Gómez-Torres 2009; González-Hernández et al. 2010) and others were selected due to observation of morphological variability among populations. The structures of 250 specimens were visualized with a Leica MZ16 stereoscope at 90×, and their frequencies were analyzed in a contingency table. We used chi-square tests to evaluate dependence among traits and study regions.

MORPHOMETRIC CHARACTERIZATION

We analyzed 10 morphometric characters using millimeters as the unit of length. We performed the following measurements: 1. ♂♀ thoracic length (TXL); 2. ♂♀ thoracic width (TXW); 3. ♂♀ abdominal length (ABL); 4. ♂♀ abdominal width (ABW); 5. ♂♀ antennal length (AL); 6. ♂ funicular setae 1 length (F1L); 7. ♂ funicular setae 2 length (F2L); 8. ♂ funicular setae 3 length (F3L); 9. ♂ funicular setae 4 length (F4L); and 10. ♂♀ total length of the body (TL). Measurements were performed with a Leica MZ16 stereoscope with a graduate lens at 90×. Antennae were photographed under the stereoscope and visualized and measured using the ImagePro Plus 4.0 software by Media Cybernetics. Data from the different traits and regions were analyzed using analysis of variance (ANOVA) and the Tukey test. Finally, we applied Principal Component Analysis (PCA) to summarize in a low number of characters the highest percentage of variation and identify groups defined by the heterogeneity of the measured characteristics; subsequently, we performed a discriminant analysis to determine differences in the group traits.

MOLECULAR ANALYSIS

DNA Isolation, Polymerase Chain Reaction, and Sequence Analysis

DNA was isolated from individual *T. radiata* wasps by the DNA extraction method suggested by the Pure Link Genomic DNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and the resultant DNA was stored at −20 °C until use. The DNA was amplified using the CI-J-1718 (forward) 5›-GGAGGATTTGGAAATTGATTAGTTCC-3› and C1-N-2191 (reverse) 5›CCCGGTAAAATTAAAATATAAACTTC-3› primers representing the *COI* partial gene (Simon et al. 1994). The polymerase chain reactions (PCRs) were performed in total volumes of 30 µL containing 1× Buffer, 2 mM each dNTP, 2 mM $MgCl_2$, 100 ng/µL each primer, and 1U/µL *Taq* polymerase; they were performed under the following conditions: 94 °C for 3 min, 40 cycles at 94 °C for 20 s, 48 °C for 20 s, 72 °C for 40 s, and a final extension step at 72 °C for 10 min. PCR products were visualized on 2% agarose gels to confirm amplification of samples and non-amplification of negative controls; then the products were sent to Macrogen Corp. (Rockville, Maryland, USA) to be sequenced in both directions. The obtained sequences were edited and aligned in MEGA 5 software (Tamura et al. 2011) and then submitted to GenBank (www.ncbi.nlm.nih.gov/genbank, accession numbers KJ995664–KJ995701). Furthermore, the number and location of variable sites and genetic distances were determined and analyzed in the same software.

In order to demonstrate intraspecific variation, we included in our alignment available sequences from GenBank (http://www.ncbi.nlm.nih.gov/) of *T. radiata* from the studies of Barr et al. (2009) and De León & Sétamou (2010). Additionally, sequences from GenBank of *Tamarixia triozae* (Burks) and *Gonatocerus metanotalis* (Ogloblin) (Hymenoptera: Mymaridae) were included as outgroups. Finally, we obtained a phylogenetic tree (neighbor-joining) to determine the genetic distance between sequences; the tree was inferred according to the Tamura-3-parameters model (Tamura et al. 2004); it was calculated with the same program (MEGA 5).

Results

MORPHOLOGICAL ANALYSIS

Nine of the 15 variables considered in the morphological analysis (Table 1) were statistically significant (chi-square test, Table 2).

Table 1. Morphological characters used for the analysis of *Tamarixia radiata* specimens from 2 Mexican geographic regions.

Character description (abbreviation)	Code
Body color (BC)	1 = dark, 2 = brown
Position of scape sensilla of males (PS)	1 = top, 2 = middle, 3 = lower, 4 = absent
Dark line on ventral side of scape of males (LE)	1 = present, 2 = absent
Length of funicular setae (LSF*)	1 = half funicular segment, 2 = 1 funicular segment, 3 = 2 funicular segments, 4 = 3 funicular segments, 5 = 4 funicular segments, 6 = absent, 7 = more than 4 funicular segments
Form of funicular length (FFL)	1 = growing form, 2 = decreased form, 3 = irregular
Number of adnotaular setae on each side (NAS)	1 = 1 seta, 2 = 2 setae, 3 = more than 2 setae
Number of propodeal callus setae (NCS)	1 = 1 seta, 2 = 2 setae, 3 = 3 setae, 4 = more than 3 setae
Spot at the beginning of the submarginal vein (SMVS)	1 = present, 2 = absent
Number of submarginal setae (SMS)	1 = 1 seta, 2 = 2 setae, 3 = more than 2 setae
Abdomen size (ABS)	1 = longer than wide, 2 = almost longer than wide, 3 = wider than long
Number of tergites covered by abdominal dorsal spot (NTS)	1 = 1 tergite, 2 = 2 tergites, 3 = 3 tergites, 4 = 4 tergites, 5 = 5 tergites, 6 = absent
Size of cerci setae (SC)	1 = as wide as final tergite, 2 = half length of final tergite, 3 = double the length of final tergite
Number of cerci setae (NCS)	1 = 1, 2 = 3, 3 = more than 3
Dark line on the tibia (DLT)	1 = all, 2 = protibia, 3 = mesotibia, 4 = metatibia, 5 = pro- & mesotibia, 6 = pro- & metatibia, 7 = meso- & metatibia, 8 = absent
Dark dorsal line on the femur (DLF)	1 = all, 2 = profemur, 3 = mesofemur, 4 = metafemur, 5 = pro- & mesofemur, 6 = pro- & metafemur, 7 = meso- & metafemur, 8 = absent

*LSF was considered from the 1st funicle.

In both geographical regions, dark specimens were more abundant than brown specimens. The highest percentage (53%) of brown males was found in the west (Colima); meanwhile, most brown females (69%) were collected in the northeast (Nuevo León) (Table 3). The presence of a dark line on the femora was different in males and females of both regions. Most of the specimens with a dark line on the femur were males (86%), whereas most of the females had legs that were entirely clear (65%). In the northeast, 40% of the females were found with this feature (Table 4) and 25% of females with darkened femora.

The proportion of the abdominal dorsal pale spot was variable. Males had a spot that covered 1 basal abdominal tergite, and the abdominal dorsal pale spot was distributed in similar proportions in both geographical regions. Females had an abdominal dorsal spot that covered approximately 3 basal tergites (Table 5), and most such specimens were found in the northeast. Commonly, the abdominal dorsal spot covers 2 tergites in males and 4 in females.

The males had antennal sensilla located either in the middle position (41%) or proximal to the base of the scape (94%); in 87% of males, the scape also had a dark line on its underside. In 57% of the specimens, the funicular setae covered 4 funicular segments, and in 38%, they covered only 3 funicular segments. The setae were observed rarely on more than 4 segments (5%). Most males (79%) had a dark spot at the beginning of the submarginal vein and 77% of males had more than 3 cerci setae. Females showed variation in body color (BC), in the presence of a dark dorsal line on the femur, and in the number of tergites covered by the abdominal dorsal spot. Furthermore, 69% of females had a telescoped funicle. Morphologically, the traits were nonspecific for any of the studied geographic areas.

MORPHOMETRIC ANALYSIS

The females featured the following morphometry: TL was 0.8–1.68 mm, TXL 0.32–0.56 mm, TXW 0.32–0.48 mm, ABL 0.32–0.80 mm, ABW 0.24–0.56 mm, and AL 0.21–0.34 mm. In males, the morphometry measurements were: TL 0.72–1.60 mm, TXL 0.32–0.50 mm, TXW 0.24–0.40 mm, ABL 0.32–0.64 mm, ABW 0.16–0.40 mm, AL 0.25–0.58 mm, F1L 0.21–0.71 mm, F2L 0.20–0.71 mm, F3L 0.20–0.68 mm, F4L 0.16–0.57 mm. Results from ANOVA and PCA showed that there were no statistically significant differences in the analyzed traits in females, and this reflected morphometric homogeneity. We found relevant differences in the males in the following characters: TL (P = 0.001, F = 5.903, df = 110), TXW (P = 0.020, F = 3.432, df = 110), ABL (P = 0.001, F = 5.889, df = 110), AL (P = 0.005, F = 4.549, df = 110). The variation was found mainly in the specimens collected in Colima State (Tukey $\alpha = 0.05$, Table 6).

According to the PCA, the size of the parasitoids had the highest percentage of variation (65%), mainly distributed in 2 principal components, PC1 and PC2. PC1 refers to the complete size of the

Table 2. Chi-square test results of morphological characters tested for differentiation of variability in *Tamarixia radiata.*

Feature	Male χ^2 (P)	Females χ^2 (P)
Body color (BC)	11.392 (0.010)	35.063 (0.000)
Number of tergites covered by abdominal dorsal spot (NTS)	17.729 (0.038)	12.574 (0.050)
Dark dorsal line on femur (DLF)	40.076 (0.000)	40.843 (0.002)
Form of funicular length (FFL)	—	19.911 (0.003)
Position of scape sensilla (PS)	41.575 (0.000)	—
Dark line on ventral side of scape (LE)	8.327 (0.040)	—
Length of funicular setae (LSF)	18.600 (0.029)	—
Spot at the beginning of the submarginal vein (SMVS)	14.210 (0.003)	—
Number of cerci setae (NCS)	23.053 (0.006)	—

Table 3. Contingency table for the frequency of body color in the analyzed specimens of *Tamarixia radiata*.

Sex	Region	State	Dark	Brown
Males	West	Colima	30	18
		Michoacán	28	1
	Northeast	Nuevo León	12	5
		Tamaulipas	32	10
	Both	All	102	34
Females	West	Colima	17	6
		Michoacán	21	0
	Northeast	Nuevo León	18	16
		Tamaulipas	52	1
	Both	All	108	23

parasitoid body, and this component represented 41% of the variation; for this component, abdominal length ABL was the morphometric characteristic that most contributed to the variation (component matrix value = 0.814) followed by the total length of the body TL (component matrix value = 0.767). PC2 denotes the size of the antennal structures. In PC2, the length of the funicular setae F3L and F4L contributed 24% to the whole amount of variation (component matrix values = 0.662 and 0.649, respectively). Although there was variation in the size of the funicular setae, the antenna did not show a statistically significant difference in its total length. The discriminant analysis showed that total length of the body TL presented most of the variation among all the examined specimens (Wilk's lambda = 0.859, $P < 0.05$).

MOLECULAR ANALYSIS

Two variable sites corresponding to transitions and transversions were identified in 413 bp fragments; such variations are the identity of *T. radiata* haplotypes H1 and H2 (Table 7). In the phylogenetic tree, each haplotype is represented by a branch (Fig. 1), and the genetic distance between the 2 branches (0.005 differences per site) indicates that both haplotypes belong to a single species (*T. radiata*), which is confirmed by comparing distances between its sister taxon *T. triozae* (0.100 differences per site). In the phylogenetic tree, the first branch represents the H2 located in the states of Colima (samples of 2010), Michoacán (samples of 2011), Nuevo León (samples of 2009 and 2010), and Tamaulipas (samples from 2006 and 2010). The 2nd branch represents the H1, which was located only in the state of Tamaulipas (samples from 2006 and 2010) and was encountered less frequently than H2 (Table 7).

Discussion

MORPHOLOGY

The morphological traits with significant differences among specimens of *T. radiata* were principally body color characters, which also have been found in previous studies (Gómez-Torres 2009; González-Hernández et al. 2010). Contrary to findings of Gómez-Torres (2009), our study demonstrated that such differences tended to be prevalent in some regions, such as the state of Colima. The highest percentage (52%) of brown males in this region could be associated with the presence of laboratory strains; in Colima, *T. radiata* is mass reared and released (Sánchez-Gonzalez 2011). In Tamaulipas (northeast), there was no record of *T. radiata* releases; however, we found a relatively high percentage (29%) of parasitoids with this distinctive characteristic. In the same northeastern region (particularly in Nuevo León), we recorded the highest percentage (69%) of brown females; thus, the possibility to use this characteristic to differentiate wild and laboratory strains by color could be inaccurate and lead to misidentifications.

The presence of a dark line on the femora was different in both sexes, and most of the specimens (86%) with this trait were males, whereas 65% of the females had pale legs. Specimens with this characteristic were concentrated in the northeast (Table 4). Variation of the gaster was also different between the sexes: most females (70%) in the northeast showed a pale spot on 3 basal tergites whereas in males, the same colored spot covered just 1 basal tergite. These males were mostly present in the west (71%). Concentration of variation in 1 of the 2 studied regions suggests the existence of a geographical differentiation of *T. radiata* in Mexico, which could be due to multiple ecological or environmental factors, such as temperature, with a direct effect on their morphology and coloration (Wagner et al. 1984; Chapman 1998; Gomez-Torres 2009; Roslin et al. 2009).

MORPHOMETRY

According to the description of the holotype (Waterston 1922), the male *T. radiata* has a TL of approximately 1.1 mm; in the studied regions, the range of length was 0.7 to 1.6 mm. TL was the trait that contributed the most to the heterogeneity in males (mainly in Colima, Table 6). Such observations were made previously by González-Hernández et al. (2010), who reported size variation in the parasitoids from diverse citrus-producing areas of Mexico. AL also differed from the holotype specimen description; for both sexes, Waterston (1922) reported a length of 0.7 mm; in our study, this size ranged

Table 4. Contingency table for the frequency of a dorsal line on femora of examined specimens of *Tamarixia radiata*.

Sex	Region	State	All	Mesofemur	Metafemur	Pro- & mesofemur	Pro- & metafemur	Meso- & metafemur	Absent
Males	West	Colima	21	0	15	0	2	4	6
		Michoacán	3	0	19	0	0	1	6
	Northeast	Nuevo León	8	1	3	1	0	0	4
		Tamaulipas	21	0	9	0	1	8	3
	Both	All	53	1	46	1	3	13	19
Females	West	Colima	5	0	2	0	0	1	15
		Michoacán	0	0	3	0	0	0	18
	Northeast	Nuevo León	4	5	1	1	1	0	21
		Tamaulipas	1	0	14	1	2	3	31
	Both	All	10	5	20	2	3	4	85

Table 5. Contingency table for the frequency of the number of tergites covered by the abdominal dorsal spot in *Tamarixia radiata*.

Sex	Region	State	One	Two	Three	Four	Five
Males	West	Colima	2	44	0	2	0
		Michoacán	3	23	3	0	0
	Northeast	Nuevo León	0	17	0	0	0
		Tamaulipas	2	39	0	0	0
	Both	All	7	123	3	2	0
Females	West	Colima	0	0	5	17	1
		Michoacán	0	0	0	21	0
	Northeast	Nuevo León	0	0	2	31	0
		Tamaulipas	0	0	10	43	0
	Both	All	0	0	17	112	1

from 0.2 to 0.3 mm in females and 0.2 to 0.5 mm in males. This variation was statistically significant only for males. ABW and ABL corroborated the variability of males, which contrasted the morphometric homogeneity of females. Similar to morphological variation, for each morphometric characteristic, there was an absence of an exclusive relationship with any of the studied regions; however, most of the variable characteristics were concentrated in Colima (west) suggesting morphometric differentiation between Colima populations. These differences could be due to phenotypic plasticity in order to mitigate the effects of environmental variation (Gómez-Torres 2009), as in the case of morphological variation.

With this study on morphology and morphometry of *T. radiata*, the possibility of differentiating populations could become practical. In order to differentiate accurately populations by this procedure, it will be necessary to increase the number of samples and areas of collection, as well as studied traits, including characters susceptible to phenotypic plasticity. Furthermore, we suggest the use of other sensitive techniques to capture most of the variation, like geometric morphometry (Rohlf & Marcus 1993; Adams et al. 2004; Slice 2007).

MOLECULAR ANALYSIS

The 2 haplotypes (H1 and H2) that we found were the same that Barr et al. (2009) reported from samples obtained during 2004 to 2008 from *T. radiata* strains brought into Florida, USA, and during 2008 from introductions in Texas, USA. In samples collected in 2006, De León & Sétamou (2010) obtained H1 in Florida and H2 in Texas; they also found H2 in samples from Sonora, Mexico. In contrast to findings reported by Barr et al. (2009), De León & Sétamou (2010) indicated that Florida, Texas, and Mexico have different haplotypes; however, it is important to note that in the study of De León & Sétamou (2010), samples from Mexican states bordering the USA were absent. González-Hernández et al. (2010) found H2 in samples obtained during the years 2008 to 2010 in 11 states of Mexico, and they found H1 to be widely distributed in Yucatan. Additionally, they found 3 haplotypes disseminated in the states of Michoacán (H5), Quintana Roo (H3), and Tabasco (H3 and H4). In contrast to information from previous works, it is clear that H1 and H2 have been present in Mexico since 2006; however, the distribution and frequency of H2 have been greater than that of H1 (Table 7, Fig. 1). The find-

Table 6. Morphometric characteristics of *Tamarixia radiata*.

		Mean (mm) ± SD (*n*)			
Region	State	TL	TXW	ABL	AL
West	Colima	1.0695 ± 0.149 (38)a	0.3579 ± 0.040 (38)b	0.4674 ± 0.073 (38)a	0.4479 ± 0.044 (38)a
	Michoacán	0.9538 ± 0.087 (26)b	0.3692 ± 0.039 (26)b	0.4154 ± 0.055 (26)b	0.4112 ± 0.033 (26)b
Northeast	Nuevo León	1.0100 ± 0.123 (16)b	0.3700 ± 0.040 (16)a	0.4350 ± 0.065 (16)b	0.4125 ± 0.061(16)b
	Tamaulipas	0.9548 ± 0.140 (31)b	0.3381 ± 0.044 (31)b	0.4052 ± 0.065 (31)b	0.4170 ± 0.048 (30)b

TL = Total body length, TXW = thoracic width, ABL = abdominal length, AL = antennae length. Values within columns followed by different letters are significantly different (Tukey test, $\alpha = 0.05$).

Table 7. Haplotypes of *Tamarixia radiata* identified in the analyzed samples showing nucleotide position, frequency, and study region.

	Nucleotide Position				
Haplotype	43	399	Number of specimens	State	Region
H1	T	G	10	Colima	West
			8	Michoacán	West
			8	Nuevo León	Northeast
			8	Tamaulipas	Northeast
H2	C	T	4	Tamaulipas	Northeast

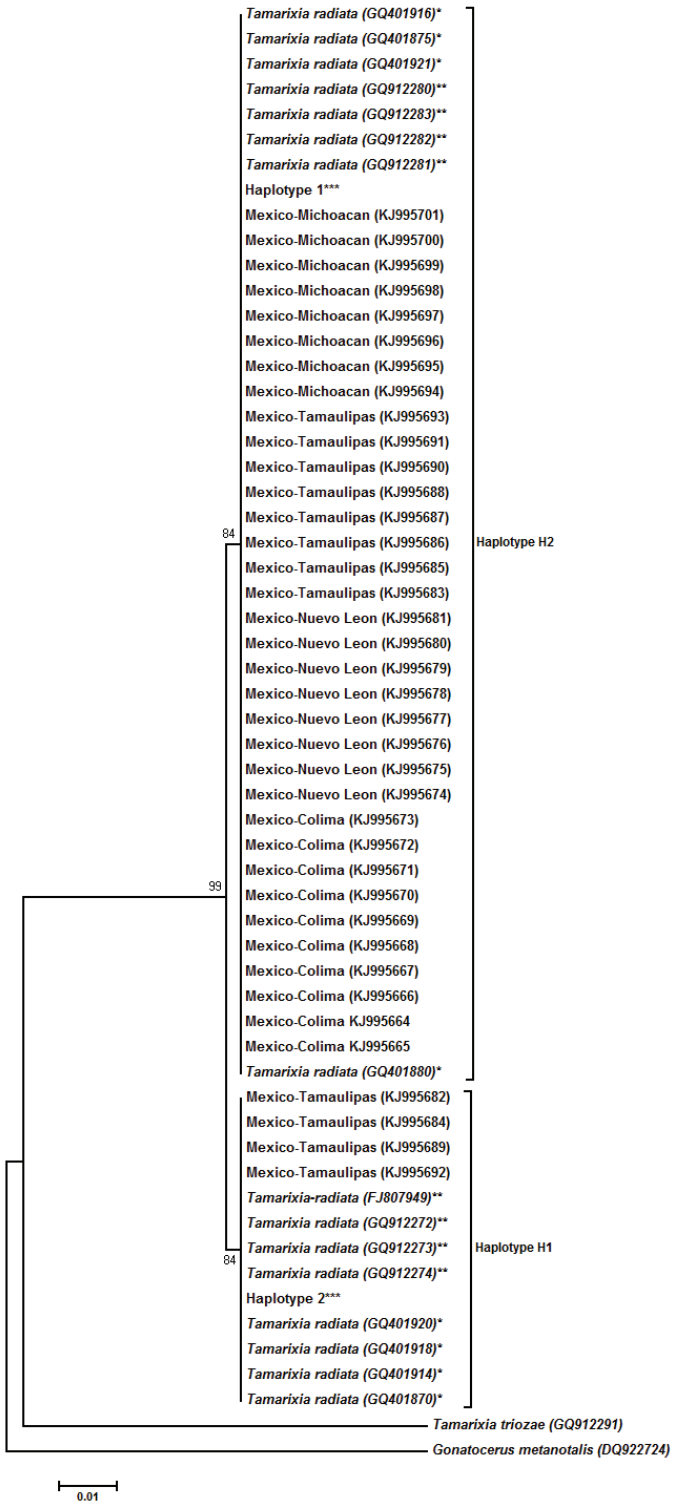

Fig. 1. Neighbor-joining tree showing the genetic distance between *Tamarixia radiata* haplotypes. The tree was inferred according to the Tamura-3-parameter model. Next to each branch appear the bootstrap values as percentage of 1,000 replications. Next to each sequence are between brackets the gene bank accession numbers; the asterisks (*) indicate the report where the sequences were obtained, i.e., *Barr et al. 2009, **De León & Sétamou (2010), and ***González-Hernández et al. (2010).

ing of H1 in 2006 in Texas, USA (De León & Sétamou 2010), and in Tamaulipas, Mexico—which are geographically next to each other—suggests the existence of gene flow between both countries, which could be increased in the short term due to the current *T. radiata* release program in Texas (Flores et al. 2014).

Among the 5 haplotypes reported in Mexico, haplotypes H1 and H2 dominated in distribution and frequency in the states of Tamaulipas and Yucatan (Fig. 2), suggesting that accidental or non-intentional introduction of the species occurred in these areas. Because *T. radiata* has been found naturally in many Mexican regions, and by 2004 was thriving in almost all the citrus-growing regions of Brazil (Gomez-Torres 2009), its wide distribution in a relatively short span of time suggests that *T. radiata* was already existing in the Americas before its introduction to the USA. Analysis of voucher specimens from the original releases of the parasitoid against *D. citri* in Guadeloupe (Étienne et al. 2001) could shed more light on a possible source of wild *T. radiata* populations that now occur in the Americas. For further studies, we suggest increasing the number of samples in order to determine the geographic scope of populations with H1, which appears to have a restricted distribution. Because *COI* is the most conserved of the mitochondrial genes in terms of amino acid evolution, and because it encodes essential functions inside the cell (Simon et al. 1994; Lunt et al. 1996), the process of fixing a mutation in this gene would be very prolonged, and the consequent ecological variations probably would not be reflected easily in it. Therefore, use of other molecular markers is necessary to corroborate whether the dispersal capability of specimens possessing H1 is limited, and to measure genetic flow at inter- and intra-population levels as a way of monitoring micro evolutionary processes.

CONCLUSIONS

Our discovery of morphological and genetic variations is evidence of the diversification process of *T. radiata* in Mexico, which could be associated with the time that this exotic species has been present in the country. However, we do not reject the possibility that the species entered Mexico exhibiting such variability. At this moment, these variations are inadequate for a practical use in the identification of geographic populations or strains. The persistent and wide distribution of the parasitoid and the dominance of certain haplotypes in some citrus-growing zones suggest that the parasitoid has been able to cope with ecological pressures in the country (Peña-Carrillo et al. 2014). Presently, the Mexican scenario where locally collected strains of *T. radiata* are mass reared and released (López-Arroyo et al. 2009; Sanchez-Gonzalez 2011) apparently provides a valuable biological control method for the management of the vector of huanglongbing (Vizcarra-Valdez et al. 2013); even so, it is necessary to determine impact on *D. citri* populations and dissemination of huanglongbing (Chen & Stansly 2014). Evaluation of success and fate of the parasitoid needs to be addressed through focused studies in the field to assess their reproductive performance, survivorship, parasitism levels, potential alternate hosts, and microhabitats, as well as possible hibernation sites and conditions for the diverse strains that are released. Such information would support the Mexican approach to control *D. citri* through the mass production and release of accidentally introduced strains of *T. radiata.* Moreover, it could help to evaluate the extent and implications of biological control programs performed elsewhere against the vector of huanglongbing, especially the pioneer program in Florida (Hoy et al. 1999; Barr et al. 2009).

Fig. 2. Distribution of *Tamarixia radiata* haplotypes H1 and H2 in Mexico.

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